Generation of disease models for neurodegenerative disorders in hESCs by gene targeting

Grant Award Details

Generation of disease models for neurodegenerative disorders in hESCs by gene targeting

Grant Type: Tools and Technologies I

Grant Number: RT1-01107

Investigator:

Name:	Ying Liu
Institution:	University of California, San Diego
Type:	PI

Disease Focus: Amyotrophic Lateral Sclerosis, Neurological Disorders

Human Stem Cell Use: Embryonic Stem Cell

Cell Line Generation: Embryonic Stem Cell

Award Value: $709,829

Status: Closed

Progress Reports

Reporting Period: Year 1

View Report

Reporting Period: Year 2

View Report

Grant Application Details

Application Title: Generation of disease models for neurodegenerative disorders in hESCs by gene targeting

Public Abstract:

The ability to target a specific locus in the mouse genome and to alter it in a specific fashion has fundamentally changed experimental design and made mice the preeminent model for studying human diseases . However, pathogenesis in humans have unique pathways that may not be revealed by only using mouse or other animal models. An approach that combines the advantages of mouse models with parallel experiments in human embryonic stem cells (hESCs) offers significant advantages over current methodologies. With the large number of hESC lines available, the ability to grow cells in defined media, the development of drug resistant feeders and the reports of strategies to insert DNA with increasing efficiency into hESC, it would only be a matter of time to obtain homologous recombinants in hESCs.

In order to provide direct clues to pathogenesis in human tissues, we propose to use homologous recombination to establish in vitro human disease models in hESCs. As a proof of principle, we have chosen Lou Gehrig's disease (or amyotrophic lateral sclerosis, ALS). ALS is a disease that progressively and selectively attacks motoneurons in the brain and the spinal cord. It becomes fatal when motoneurons controlling breathing are affected. Approximately 2% of ALS cases have been identified to be caused by mutations of the the Cu-Zn superoxide dismutase (SOD1) gene in an autosomal dominant trait. Animal models have been established and researchers have been able to propose disease mechanisms which led to potential treatments, although no cure has been offered yet. This in part might be due to lack of human cell based models and varied mutant copy numbers in transgenic animals as well as the random nature of their integration into the genome.

Here, we propose to generate hESC lines by gene targeting to harbor point mutations in the SOD1 gene, which recapitulates the genetic defects in SOD1 mutated ALS patients. We will further target these mutations in hESC reporter lines of the two important cell types in ALS: motoneurons and astrocytes. The availability of these SOD1 mutated hESC and hESC reporter lines will allow researchers to obtain purified “diseased” motoneurons and astrocytes, which will facilitate the dissection of ALS pathogenesis. The completion of this proposal will provide (1) a highly efficient protocol for performing homologous recombination in hESCs, (2) a package of motoneuron and astrocyte reporters which are useful for both disease and developmental studies along the neural lineages, and (3) a set of ALS disease platforms of hESC lines to serve as an hESC ALS disease in vitro model, as well as a virtually unlimited source of “diseased” motoneurons and astrocytes. This work not only will provide tools to move pathogenesis research for ALS, but also can be reliably extended into other neural and non-neural lineage diseases, of which genetic defects have been identified, including Huntington's disease (HD) and Parkinson's disease (PD).

Statement of Benefit to California:	The overall objectives for this proposal are to create in vitro human neurodegenerative disease models using human embryonic stem cells (hESCs), and as a proof of principle, three point mutations of the SOD1 gene which cause familial amyotrophic lateral sclerosis (FALS) will be tested first. These SOD1 missense mutations, G37R, G85R and G93A, have been identified in FALS patients and widely used in rodent models of FALS. We propose to create SOD1 mutations in hESC lines by gene targeting technology which has been proven to be revolutionary in establishing disease models in animals. In addition, we will use similar protocol to generate motoneuron and astrocyte reporter lines in hESCs, since these two cell types and the interaction between them play the most critical roles in the pathogenesis of ALS. After obtaining the three SOD1 missense mutants in motoneuron and astrocyte reporter lines, we will extend our efforts to characterization of these lines, by examining their growth, survival, cell death and other biochemical properties. We will also perform large scale comparisons for genomic and proteomic profiles of the diseased hESC lines with wild type hESCs, as well as comparing the “diseased” and wild type hESC-derived populations of motoneurons and astrocytes. These experiments will not only provide direct clues for ALS pathogenesis research but also serve as a proof of principle for general disease research using hESCs as a model system. The protocols and reagents developed in this work will be available for Californian researchers and physicians to use for similar neurodegenerative diseases or diseases of other systems. This work will eventually facilitate the scale-up in establishment of human diseases models using human tissues or human cell culture systems for our colleagues in California and around the world.